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A simple and inexpensive technique for assessing microbial contamination during drilling operations

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Exploration of the Deep Biosphere relies on drilling, which inevitably causes infiltration of drilling fluids, containing allochthonous microbes from the surface, into the core. Therefore it is absolutely necessary to trace contamination of the sediment core in order to identify uncontaminated samples for microbiological investigations. Several techniques have been used in the past, including fluorescent dyes, perfluorocarbon tracers and fluorescent microspheres. Fluorescent dyes are inexpensive and easy to analyze on-site but are sensitive to light, pH and water chemistry. Furthermore, significant sorption to clays can decrease the fluorescence signal. Perfluorocarbon tracers are chemically inert hydrophobic compounds that can be detected with high sensitivity via gas chromatography, which might be a problem for on-site analysis. Samples have to be taken immediately after core retrieval as otherwise the volatile tracer will have diffused out of the core. Microsphere tracers are small $(0.2 - 0.5 \, \mu m$ diameter) fluorescent plastic particles that are mixed into the drilling fluid. For analysis, these particles can be extracted from the sediment sample, transferred onto a filter and quantified via fluorescence microscopy. However, they are very expensive and therefore unsuitable for deep drilling operations that need large amounts of drilling fluids.

Here, we present an inexpensive contamination control approach using fluorescent pigments initially used for coloring plastics. The price of this tracer is nearly three orders of magnitude lower than conventional microsphere tracers. Its suitability for large drilling campaigns was tested at the ICDP Deep Drilling at Lake Towuti, Sulawesi, Indonesia. The tracer was diluted 1:1000 in lake water, which was used as the drilling fluid. Additionally, a plastic bag filled with 20 mL of undiluted tracer was attached to the core catcher to increase the amount of particles in the liner fluid right at the core. After core retrieval, the core was cut and the liner fluid collected. From each whole round core (WRC) that was taken for microbiological and biogeochemical analyses, small samples of 1 cc were retrieved with sterile cutoff syringes from the rim, the center and an intermediate position. After dilution and homogenization in 9 mL MilliQ water, $10~\mu$ L of the sediment slurry was transferred onto a filter membrane and particles counted via fluorescence microscopy. Additionally, particles in the liner fluid were also quantified. This allows the quantification of the amount of drilling fluid that has entered the sediment sample during drilling. The minimum detectable volume of drilling fluid was in the order of single nanoliters per cc of sediment, which is in the range of established techniques.

The presented method requires only a minimum of equipment and allows rapid determination of contamination in the sediment core and an easy to handle on-site analysis at low costs. The sensitivity is in the same range as perfluorocarbon and microsphere tracer applications. Thus, it offers an inexpensive but powerful technique for contamination assessment for future drilling campaigns.